DETAILED ACTION

Continued Examination Under 37 CFR 1.114

A request for continued examination under 37 CFR 1.114, including the fee set forth in 37 CFR 1.17(e), was filed in this application after final rejection. Since this application is eligible for continued examination under 37 CFR 1.114, and the fee set forth in 37 CFR 1.17(e) has been timely paid, the finality of the previous Office action has been withdrawn pursuant to 37 CFR 1.114. Applicant's submission filed on 2/21/2008 has been entered.

Claim Status

Claims 1-21 are pending. Claims 1-21 are under current examination.

Priority

This application claims benefit from provisional U.S. Application No. 60/559,209, filed 1 April 2004. The instant application has been granted the benefit date, 1 April 2004, from the application 60/559,209.

Response to Arguments - Claim Rejections 35 USC § 103

The rejection of claims 1-21 under 35 U.S.C. 103(a) as being unpatentable over Thorey et al. (Molecular and Cellular Biology. May 1998. Vol.18, No.5: 3081-3088) in view of Zambrowicz et al. (Int.J.Dev.Biol. 1998; 42: 1025-1036) and further in view of Velculescu et al. (Science. 20 October 1995: 484-487) and further in view of Wan et al. (Journal of Molecular Endocrinology. 2002. 28: 177-192) is withdrawn because of the applicant's arguments.

Applicant's arguments (Remarks, pages 7-8) filed 21 February 2008 have been fully considered and they are persuasive.

The applicant argues that the Wan reference does not remedy the deficiencies in the teachings of Thorey et al., Zambrowicz et al., and Velculescue et al. related to a cell lineage targeting vector comprising…a cell lineage specific gene promoter. The applicant emphasizes that the promoter trap targeting vectors of Wan et al. do not comprise hormone or tissue specific promoters. Because of applicant's presentation, the examiner has reexamined his reasoning for introducing the Wan et al. reference. While the Wan et al. reference does refer to provirus promoter trap structures that mirror the claimed targeting vector, the examiner concurs with the applicant's assessment that the teachings of Wan et al. do not actually provide the additional teachings to make obvious a targeting vector comprising cell lineage specific gene promoter upstream of the recombinase recognition sites.

Therefore, the examiner hereby withdraws the rejection of claims 1-21 under 35 U.S.C. 103(a) as being unpatentable over Thorey et al. (Molecular and Cellular Biology.

May 1998. Vol.18, No.5: 3081-3088) in view of Zambrowicz et al. (Int.J.Dev.Biol. 1998; 42: 1025-1036) and further in view of Velculescu et al. (Science. 20 October 1995: 484-487) and further in view of Wan et al. (Journal of Molecular Endocrinology. 2002. 28: 177-192).

NEW GROUNDS OF REJECTION

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claims 1-21 are rejected under 35 U.S.C. 103(a) as being unpatentable over Thorey et al. (Molecular and Cellular Biology. May 1998. Vol.18, No.5: 3081-3088) in

view of Zambrowicz et al. (Int.J.Dev.Biol. 1998; 42: 1025-1036) and further in view of Velculescu et al. (Science. 20 October 1995: 484-487) and further in view of Nagy (Genesis 2000; 26:99-109).

The claimed invention is directed to a method for identifying genes expressed during differentiation of a cell comprising the steps of: a) integrating into a site in the genome of a host cell, a cell lineage targeting vector comprising, a pair of recombinase recognition sites flanking one or more polyadenylation sites, a first selectable marker placed downstream of or between the two recombinase recognition sites, a reporter gene placed downstream of the recombinase recognition sites, and a cell lineage specific gene promoter placed upstream of the recombinase recognition sites or a cell specific lineage gene placed downstream of the recombinase recognition sites, b) amplifying cells generated from the host cell; c) integrating into the genome of a plurality of the amplified cells, a gene-trap vector comprising a splice acceptor, a type IIS restriction endonuclease cleavage site, a recombinase, one or more polyadenylation sites, a second selectable marker and a splice donor; d) allowing the cells to differentiate; e) isolating cells in which the reporter gene is expressed indicating expression of the cell lineage specific gene; f) identifying trapped genes in the isolated cells. The claimed invention also incorporates elements of modified serial analysis of gene expression (SAGE), particularly type IIS endonuclease sites and Assay Tags.

The teachings of Thorey et al. are summarized below:

Thorey et al. teach, “a strategy employing gene trap…and site-specific recombination (Cre.loxP) has been used to identify genes that are transiently expressed

during…development. Thorey et al. teach diagrams (Fig.1, page 3082), that show a system of two vectors. One of the vectors comprises a pair of recombinase recognition sites (loxP) flanking one polyadenylation site, a selectable marker (Neo) placed between the recombination sites, a reporter gene (lacZ) downstream of the recombination sites. The other vector comprises cellular promoter and a trapped gene flanked by recombination recognition sites (Cre). Thorey et al. describe isolating cells derived using their system (pages 3082-3083). Thorey et al. teach, that their system is "useful for demarcating cell lineages and for tracking cell fate and migration in the developing embryo." (page 3087).

Claim 2 is directed to the method of claim 1 wherein the trapped genes are sequenced. Thorey et al. teach sequencing of genes, using plasmids (concatamers).

Claim 3 is directed to the method of claim 2, wherein inverse PCR is used. Thorey et al. teach "inverse PCR" (page 3082).

Claim 4 is directed to the method of claim 2, wherein RT-PCR is used. Thorey et al. utilize rapid amplification of cDNA ends (RACE). Accordingly, the cDNA was derived from RNA and needed to be reverse transcribed.

Claim 5 is directed to the method of claim 1, wherein the step of identifying the trapped genes in step f) comprises the steps of: a) preparing mRNA from cells in which the fluorescent reporter is expressed in d); b) synthesizing a first and second cDNA strands from the mRNA; c) digesting with type IIS restriction endonucleases to produce Assay Tags wherein each Assay Tag comprises a portion of a trapped gene and a portion of the gene-trap vector; d) concatenating the Assay Tags; e) amplifying and

sequencing the concatamers to identify the sequence of the portion of the trapped gene. Thorey et al. teach, selection of clones, isolation of cellular RNA, followed by RACE (page 3084, footnotes of Table 1). These steps comprise the sequence strategy of Thorey et al. According to Thorey et al. "all sequences showed typical cell DNA-provirus junctions" (page 3085), indicating that the region sequenced contained both the trapped sequence and the gene-trap vector.

Claim 10 is directed to the method of claim 1, wherein the recombinase is Cre or FLP. Thorey et al. teach Cre recombinase.

Thorey et al. does not specifically teach use of the SAGE technique with gene trapping. Furthermore, Thorey et al. does not teach targeting vectors comprising gene promoter placed upstream of the recombinase recognition site.

Zambrowicz et al teach however, teach the use of SAGE with gene trapping techniques (page 1026). Zambrowicz et al. discuss the usefulness of combining gene trap and SAGE techniques for "high through-put methods…used for studying expression patterns of large numbers of genes at the RNA [level]." (page 1026) In particular, Zambrowicz et al. describe the utility of gene traps for identifying genes expressed during differentiation. Zambrowicz et al. also teach alternative selection techniques comprising the use of thymidine kinase fusion proteins (page 1030, col.1), satisfying the claim limitations of claims 11 and 21.

The details of SAGE are discussed in Velculescu et al., including the use of type II restriction sites and assay tags. Velculescu et al. also teach biotinylated DNA, as per the limitations of claims 6 and 16.

Nagy teaches methods of creating transgenic mice using cell lineage specific conditional expression of Cre recombinase (page 101, col.2, lines 34-39 and page 103, col.1, lines 1-3, 8-11). Figure 2 (page 102) shows a targeting vector structure in which a tissue specific promoter is upstream of Cre recombination sites and in which a selectable marker is between two Cre recombination sites. Further Nagy teaches that their methods could be used for "phenotype analyses or…[where] a lineage- or cell type-restriction, could serve as an informative tool" (page 105, col.1, lines 17-20). Further, Nagy teaches combining their tissue specific Cre recombinase expression methods with gene trap methologies (page 108, col.1, lines 13-14, 30-34).

It would have been obvious to combine the cell lineage-specific promoter Cre-lox methods of Nagy with the systems of Thorey et al. and Zambrowicz et al. and Velculescu et al. to produce a method for identifying genes expressed during differentiation of a cell. It would have been obvious to the person of ordinary skill in the art at the time the invention was made to combine the use of gene traps and serial analysis of gene expression. In addition, a skilled artisan would find it obvious to utilize an enhanced green fluorescent protein (as in claims 8-9 and 18-19) as a functional equivalent of β-galactosidase in the systems described by Thorey and Zambrowicz.

The person of ordinary skill in the art would have been motivated to make those modifications because "gene trapping combined with methods to monitor induction of expression of the trapped gene have now been used in a variety of cell types" (Zambrowicz, page 1030) and Zambrowicz et al. suggest combining gene trap and SAGE techniques for "high through-put methods…used for studying expression patterns

of large numbers of genes at the RNA [level]." (page 1026). Furthermore, combining high throughput screening elements with gene trapping vectors is merely "making integral" the two known processes; the MPEP 2144.04 holds that this type of combination is obvious. The person of ordinary skill in the art would have been motivated to make those modifications because fluorescent proteins, such as Green Fluorescent Protein (GFP) are functionally equivalent to lacZ (β-galactosidase) systems and do not require further reagents, such as X-gal, for visualization, as is the case for β-galactosidase. Furthermore, Nagy suggests combining cell lineage-specific promoter Cre-lox systems with gene trap methods. In addition, Nagy suggests that their methods could be used as an informative tool for lineage-specific phenotypic analysis.

The skilled artisan would have had a reasonable expectation of success in combining the teachings of Thorey and Zambrowicz and Velculescu and Nagy because each of these teachings generated successful results independently and there is no indication that the combinations of high throughput screening elements with gene trapping vectors would be unsuccessful.

Therefore the method as taught by Thorey et al. in view of Zambrowicz et al. and further in view of Velculescu et al. and further in view of Nagy would have been *prima facie* obvious over the method of the instant application.

Conclusion

No claims are allowed.

Examiner Contact Information

Any inquiry concerning this communication or earlier communications from the examiner should be directed to **Scott Long** whose telephone number is **571-272-9048**. The examiner can normally be reached on Monday - Friday, 9am - 5pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, **Joseph Woitach** can be reached on **571-272-0739**. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free).

/SDL/ Scott Long Patent Examiner, Art Unit 1633	/Janet L. Epps-Ford/ Primary Examiner, Art Unit 1633